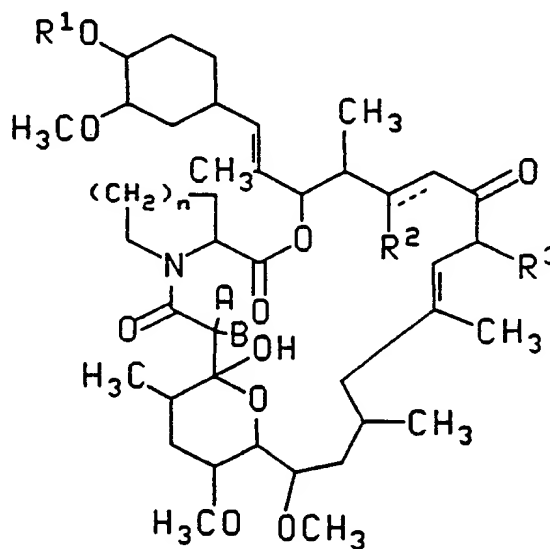




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(54) Title: SUGAR DERIVATIVES OF MACROLIDES



(I)

(57) Abstract

Macrolides of formula (I) and methods of treatment of resistance to transplantation, fungal infections and autoimmune diseases such as rheumatoid arthritis and psoriasis using said macrolides of formula (I). Wherein n is 1 or 2; A and B are taken together and form =O or A and B are taken separately and are each H or A is OH and B is H; R¹ is a glycosyl group; R² is OH or a glycosyloxy group; and R³ is an alkyl or allyl group.

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SUGAR DERIVATIVES OF MACROLIDESBackground of the Invention

This invention relates to new chemical compounds which have value in the field of medical science. More particularly, it relates to new chemical compounds which are of value for administration to a mammalian subject, particularly man, as immunosuppressive agents. These new immunosuppressive agents can be compared to the macrolides known as FK-506 and FK-520, which are described in further detail in United States Patent No. 4,894,366. The new compounds of this invention will find special utility in preventing or treating graft rejection following skin or organ transplant surgery and in preventing or treating autoimmune diseases such as rheumatoid arthritis and psoriasis. Additionally, these macrolide derivatives will find use in preventing or treating infectious diseases caused by fungi.

Graft or organ transplant rejection following transplant surgery is a common occurrence which arises when foreign antigens are recognized by the host's immune response system. The host's immune response system, in an effort to "protect" itself from the foreign tissue, then releases its cellular and humoral arsenal. The antibodies attack the foreign tissue, resulting in complications which often end in rejection of said tissue.

Similarly, the occurrence of immunoregulatory irregularities in autoimmune and chronic inflammatory diseases is well known. Irrespective of the underlying etiology of the condition, a variety of autoantibodies and self-reactive lymphocytes often arise to complicate the condition.

Treatments which target the immune response system often result in a complete shutdown of the system, leading to a lowering of the body's ability to combat infection. This can be as dangerous as the original condition which led to the shutdown.

Currently the leading medicinal agent for the prevention or treatment of graft rejection is cyclosporin A, approved by the United States Food and Drug Administration in 1983. The drug acts by inhibiting the body's immune response system from mobilizing its arsenal of natural protecting agents to reject the transplant's foreign protein. Although cyclosporin is effective in fighting graft rejection, it suffers drawbacks in that it can cause kidney failure, liver damage and ulcers; which in many cases can be very severe. Safer drugs which are more selective in their ability to affect the

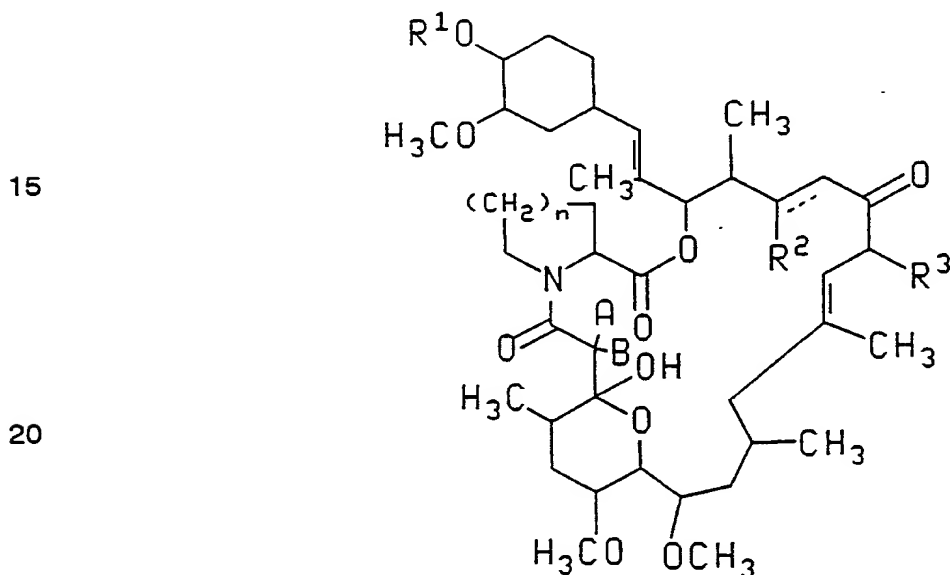
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immune response system and which have fewer side effects are constantly being pursued.

United States Patent No. 4,894,366 discloses the macrolides FK-506 and FK-520, *inter alia*, as immunosuppressants, including the treatment of "resistance to
5 transplantation," autoimmune diseases and infectious diseases. International Patent Publication No. WO 91/02736 discloses derivatives of FK-506, FK-520 and related macrolides. European Patent Publication No. 428,365 A1 discloses various other derivatives of FK-506, FK-520 and related macrolides.

Summary of the Invention

10 The present invention is directed to compounds of the formula



(I)

or a pharmaceutically acceptable salt thereof;

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wherein n is 1 or 2;

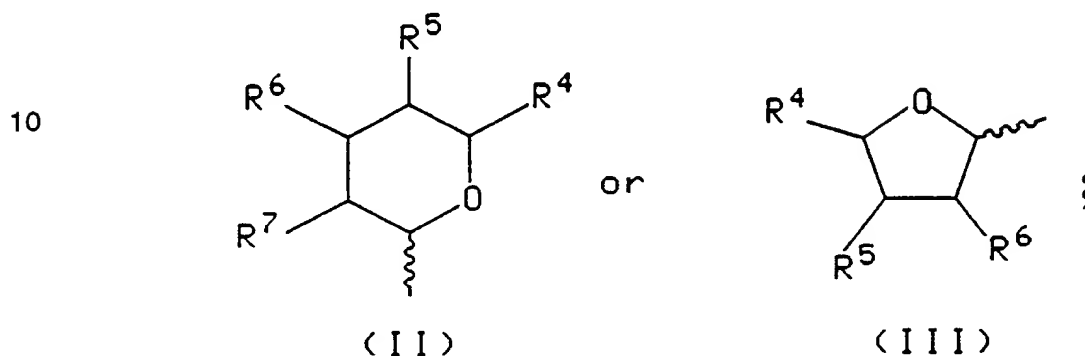
the dotted line represents an optional double bond in the case where R² is H;

A and B are taken separately and A is H and B is H or OH, or A and B are taken together and form =O;

5 R² is H, (C₂-C₅)alkanoyloxy or -OR⁰;

R³ is (C₁ to C₃)alkyl or allyl;

R¹ and R⁰ are each H,



R⁴ is, for each occurrence, independently -CO₂R⁸, -CO₂H, -CH₂OH, H, -CH₃, -CONH₂, -CONHR⁸, -CONR₂⁸, -CH₂OCOR⁸, -CH₂OCO₂R⁸, -CH₂OCONHR⁸, -CH₂OCONR₂⁸ or -CH₂OR⁸;

20 R⁵, R⁶ and R⁷ are, for each occurrence, independently (C₁ to C₄)alkoxy, benzyloxy, -OH, -OCOR⁸, -OCO₂R⁸ or -OSi(R⁹)₃;

R⁸ is (C₁-C₆)alkyl, (C₃-C₆)cycloalkyl, allyl, pyridyl, thienyl, benzyl, benzyl variously substituted with one to five halogen atoms, -OH groups or (C₁-C₄)alkoxy groups, phenyl or phenyl variously substituted with one to five halogen atoms, -OH groups or (C₁ to C₄)alkoxy groups; and

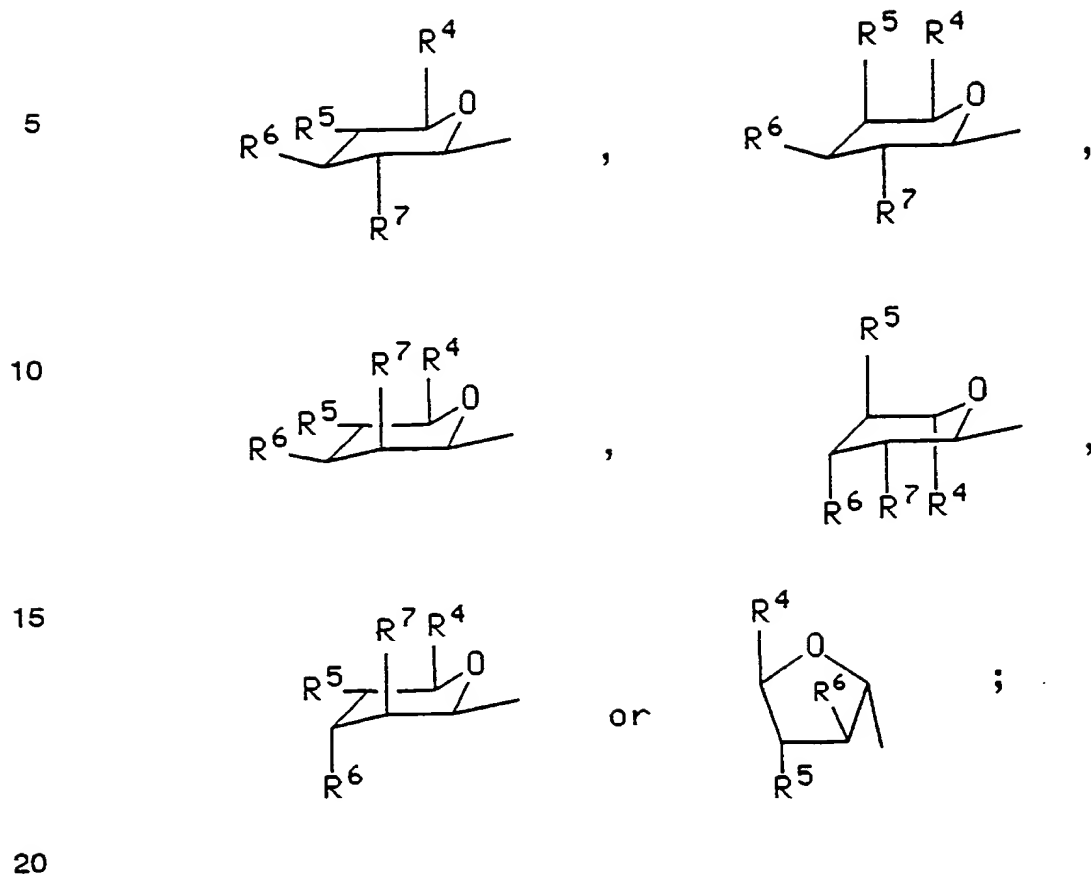
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R⁹ is, for each occurrence, independently (C₁ to C₄)alkyl, phenyl or benzyl; provided that R¹ and R⁰ are not both H.

A preferred group of compounds of this invention is the group of compounds of formula (I) wherein the dotted line represents no bond and R² is -OH.

30 A more preferred group of compounds of this invention within the preferred group of compounds are those compounds wherein n is 2; the dotted line represents no bond; A and B are taken together and form =O; R² is -OH; R³ is ethyl; R¹ is

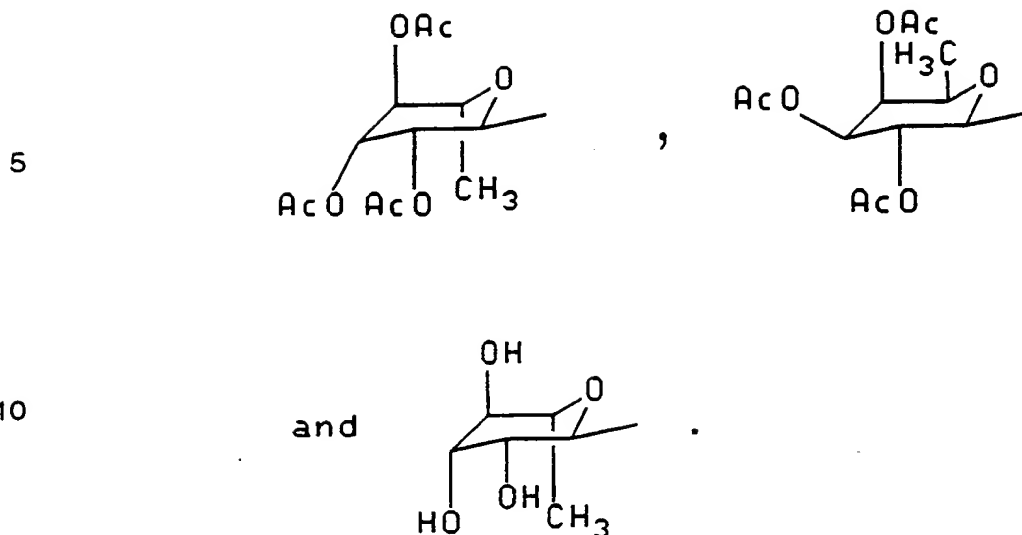
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R^4 is $-\text{CH}_2\text{OH}$, $-\text{CH}_3$, $-\text{CH}_2\text{OCOCH}_3$, $-\text{CH}_2\text{OCOCH}_2\text{C}_6\text{H}_5$ or $-\text{CO}_2\text{CH}_3$; and R^5 , R^6 and R^7 are each, independently, $-\text{OH}$, $-\text{OCOCH}_3$ or $-\text{OCOCH}_2\text{C}_6\text{H}_5$.

Especially preferred within this group are the compounds where R^1 is

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15 A second preferred group of compounds of this invention is the group of compounds of formula (I) wherein n is 2; A and B are taken separately and are each H; the dotted line represents no bond; R² is -OH; and R³ is ethyl.

The compounds of formula (I) are active as immunosuppressants. This activity makes these compounds useful in treating and preventing graft and transplant rejection. Further, this activity makes these compounds useful in preventing and
20 treating autoimmune diseases such as rheumatoid arthritis and psoriasis in a mammal, especially man.

Accordingly this invention also embraces a method of treating resistance to transplantation in a mammal in need of such treatment comprising administering to said
25 mammal a compound of formula (I) or a pharmaceutically acceptable salt thereof.

The term "transplantation," when used above and hereinafter, refers to the implantation in one part of an individual of a tissue or organ taken from another part of that individual or from another individual. Typical transplantations include, but are not limited to, bone marrow, heart, renal, tendon and pancreaticoduodenal
30 transplantations.

The term "graft" when used above and hereinafter, refers to any unattached tissue or organ which is used for transplantations. Typical grafts include, but are not limited to, skin, bone, fat and nerve grafts.

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Additionally this invention embraces a method of treating autoimmune disease (such as rheumatoid arthritis or psoriasis) in a mammal in need of such treatment comprising administering to said mammal a compound of formula (I) or a pharmaceutically acceptable salt thereof.

- 5 Further, this invention embraces a pharmaceutical composition comprising a resistance to transplantation treating effective amount of a compound of formula (I) and a pharmaceutically acceptable carrier.

- Still further this invention embraces a pharmaceutical composition comprising an autoimmune disease (such as rheumatoid arthritis or psoriasis) treating effective
10 amount of a compound of formula (I) and a pharmaceutically acceptable carrier.

Yet further the compounds of this invention of formula (I) have antifungal activity. Hence these compounds can be used to treat or prevent infections in mammals caused by fungi.

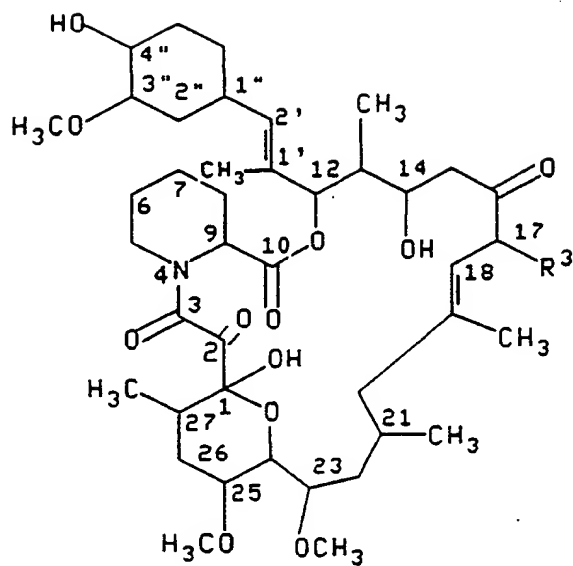
- Accordingly, this invention embraces a method of treating diseases caused by
15 fungi in a mammal in need of such treatment comprising administering to said mammal an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

- Additionally, this invention embraces a pharmaceutical composition comprising a fungal infectious disease treating effective amount of a compound of formula (I) and
20 a pharmaceutically acceptable carrier.

Detailed Description

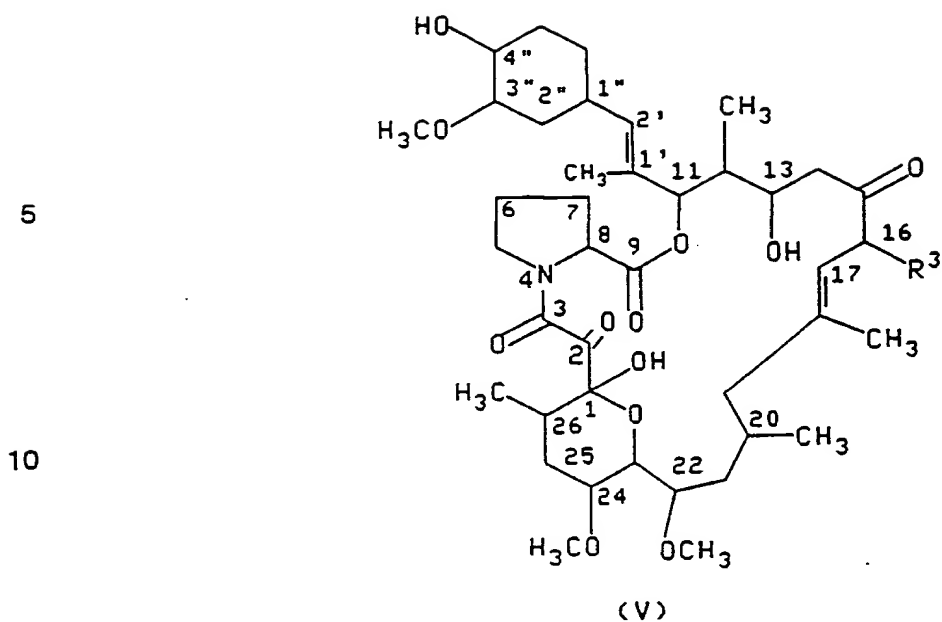
The compounds of formula (I) of the present invention are readily prepared. Most generally, a macrolide of formula (IV) or (V) below

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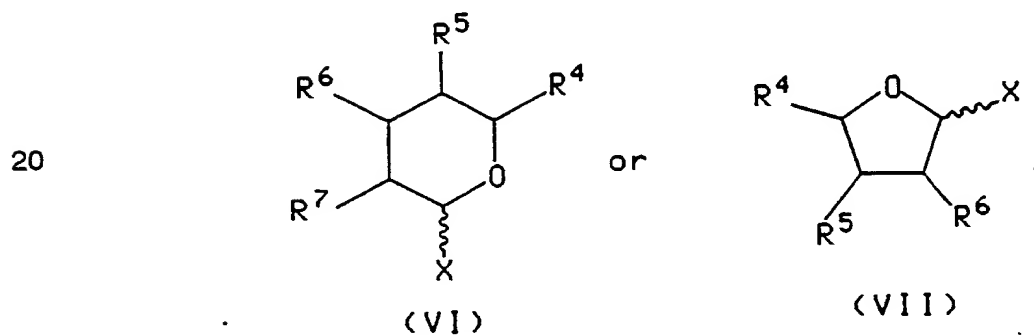
(IV)

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is coupled with an appropriate sugar halide derivative of the formula



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30 wherein X is halo, such as bromo or chloro. The coupled (or glycosylated) macrolide is then further modified as described hereinbelow.

The production of macrolides of formulae (IV) and (V) is well-known in the literature. The generally preferred route to these macrolides is *via* biological

fermentation of microorganisms belonging to the genus *Streptomyces*. The compounds of formulae (IV) and (V) wherein R³ is allyl are obtained by fermentation of *Streptomyces tsukubaensis* No. 9993 (Ferm BP-927). The compound of formula (IV) wherein R³ is ethyl and the compound of formula (IV) wherein R³ is methyl are obtained
5 by fermentation of *Streptomyces hygroscopicus* subsp. *ascomyceticus* ATCC 14891.

A lyophilized sample of *Streptomyces hygroscopicus* subsp. *ascomyceticus* ATCC 14891 has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A., under the terms of the Budapest Treaty on January 13, 1992. This newly deposited culture was given the new deposit
10 number of ATCC 55276.

Streptomyces tsukubaensis No. 9993 (Ferm BP-927) is currently on deposit with the Fermentation Research Institute, Agency of Industrial Science and Technology (No. 1-3, Higashi-1-chome, Yatabemachi, Tsukuba-gun, Ibaraki Prefecture, Japan), under the provisions of the Budapest Treaty. A fresh sample of the microorganism will
15 be deposited with the American Type Culture Collection in accordance with the terms of the Budapest Treaty.

The above-mentioned microorganisms, when placed separately in aqueous nutrient media, will produce the aforementioned compounds of formulae IV and V. The fermentation of said microorganisms to produce these macrolides is accomplished
20 substantially as disclosed in U.S. Patent 4,894,366, which is hereby incorporated by reference. Any changes made to the disclosed procedure are made in order to accommodate existing equipment at the facility and are described in Preparations 1 and 2 hereinbelow.

To prepare the compound of formula (I) wherein R⁰ is H and R¹ is a sugar substituent of formula (II) or (III), a macrolide of formula (IV) or (V) is coupled with a
25 sugar halide of formula (VI) or (VII).

The coupling (or glycosylation) reaction of a sugar halide of formula (VI) or (VII) and a macrolide of formula (IV) or (V) is accomplished in a straightforward manner, using chemistry well known to one of ordinary skill in the art. The coupling reaction is
30 generally carried out using the peracetylated form of the sugar. About 2-4 molar equivalents of the appropriate sugar halide of formula (VI) or (VII) is mixed with the macrolide of formula (IV) or (V) in a reaction inert solvent. Reaction inert solvents useful for this type of reaction include chlorinated solvents such as chloroform, methylene

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chloride and ethylene dichloride; ether solvents such as diethyl ether, tetrahydrofuran, dioxane and dimethoxyethane; aromatic solvents such as benzene, toluene and xylene; and dipolar aprotic solvents such as N,N-dimethylformamide, acetonitrile and N-methylpyrrolidone. Preferred solvents are chlorinated solvents and a particularly preferred solvent is methylene chloride. Generally it is desirable to employ enough solvent such that the reactants are dissolved or suspended by the solvent. Typically the amount of solvent used is varied to give a 10^{-1} to 10^{-3} Molar solution of macrolide with 10^{-1} Molar being preferred. Dry conditions are maintained during the course of the reaction by the utilization of anhydrous solvents and by the addition of a drying agent to the reaction mixture. Drying agents typically used for this purpose are molecular sieves, calcium sulfate and magnesium sulfate. A preferred drying agent is 4Å molecular sieves. Initial mixing of the reagents is performed at a temperature of from about -78°C to about 70°C . Preferred are temperatures ranging from about -78°C to about 0°C . Especially preferred for ease of preparation is a cooling bath which maintains the reaction temperature at -78°C .

After the above-mentioned reactants have been mixed and the temperature has equilibrated to -78°C , the reaction mixture is treated with a suitable base such as mercuric carbonate, silver carbonate, mercuric nitrate or silver nitrate. The preferred base for this reaction is silver carbonate. Following addition of said base, the reaction mixture is treated with a catalyst. Typical catalysts for this reaction include triflate, perchlorate and tetrafluoroborate salts of the cation associated with the particular base used. The preferred catalyst is silver triflate.

After all reactants and reagents have been added, the reaction mixture is warmed to 0°C , stirred for 0.5-24 hours at 0°C and then warmed slowly to room temperature. The reaction mixture is stirred for an additional 0.5-24 hours at room temperature. Generally, the reaction mixture is stirred at 0°C for 5 hours and allowed to warm to room temperature over 3 hours followed by stirring at room temperature for 16 hours. The product is then isolated from the reaction mixture using techniques familiar to one of ordinary skill in the art. Thus, simple filtration through a filter aid such as Celite[®] followed by evaporation affords a residue which is purified by column chromatography. One of ordinary skill in the art will recognize that column chromatography entails the use of a solid phase component such as silica gel and a liquid phase component comprised of an advantageous mixture of solvents for the

separation and purification of compounds from a mixture. Removal of solvents after chromatography affords the glycosylmacrolide.

Generally, the coupling reaction only takes place at one of the three alcohol sites of the macrolide, this site being the C-4" alcoholic functionality (see Formula IV).

5 This selectivity is possibly due to the greater availability of the hydroxyl group of this position in the macrolide's preferred conformation. On occasion, however, with particularly reactive sugar halides, small amounts of diglycosylated material (wherein $R^2 = OR^0$) are formed. This material is detected during the monitoring of the progress of the reaction, which is generally accomplished *via* thin layer chromatography, according to
10 standard practice. The diglycosylated material is isolated and purified as for the monoglycosylated material with the notable exception that the diglycosylated material is the first material isolated from the chromatography, with the monoglycosylated material being isolated in later fractions. The use of added equivalents of sugar chloride or the altering of other parameters such as solvent, base or catalyst can affect
15 the yield of diglycosylated material.

To prepare the compound of formula (I) wherein R^1 is H and R^0 is a sugar substituent of the formula (II) or (III), a macrolide of formula (IV) or (V) is first protected with a hydroxyl protecting group at the C-4" position. Hydroxyl protecting groups suitable for such purposes include but are not limited to such groups as silyl ethers,
20 carboxylic esters and carbonic esters of the alcohol. The protecting groups are appended to the alcohol utilizing the well known methods of organic chemistry. Bulky silyl ethers are preferred for their selectivity, ease of attachment and ease of removal. Conveniently, a macrolide of formula (IV) or (V) is dissolved in a reaction inert solvent at a temperature of about 0°C to about 30°C. Reaction inert solvents for this type of
25 reaction include dipolar aprotic solvents such as dimethylformamide, acetonitrile and N-methyl pyrrolidone; chlorinated solvents such as chloroform, dichloromethane and 1,2-dichloroethane; and ether solvents such as diethyl ether, dioxane and tetrahydrofuran. The solvent of choice is often dimethylformamide. A silylating agent, usually a silyltrifluoromethanesulfonate such as t-butyltrimethylsilyltrifluoromethanesulfonate or a silyl chloride such as dimethyl-t-butylsilyl chloride,
30 trimethylchlorosilane or triphenylchlorosilane, is added along with an organic amine such as triethylamine, trimethylamine or imidazole. Ordinarily imidazole is the preferred base. The reaction mixture is stirred for about one hour to about 24 hours, typically at

room temperature, after which time the product is isolated from the reaction broth in a manner well known to one of ordinary skill in the art.

The macrolide, now protected at the C-4" position, can be coupled with a sugar halide of formula (VI) or (VII) as described hereinabove. The product of such a coupling reaction is a derivative of a compound of formula (I) with a sugar derivative
5 attached by way of oxygen to the C-14 position and with a protected C-4" position. The C-4" position can be deprotected to afford the free hydroxy compound by employing standard methods of organic chemistry well known to one of ordinary skill in the art. Typically, to remove a preferred silyl ether protecting group, the C-4"-silyl protected
10 compound of formula (I) is dissolved in a reaction inert solvent such as acetonitrile or an ether solvent such as tetrahydrofuran or diethyl ether at a temperature of about 0°C to 30°C and is treated with a fluoride source such as hydrogen fluoride or tetra-N-butylammonium fluoride. The reaction is stirred for about one hour to about 24 hours and the product is then isolated by employing standard methods of organic chemistry
15 well known to one of ordinary skill in the art.

To prepare the compounds of the invention of formula (I) wherein A and B are taken separately and are each H (hereinafter referred to as the C-2 desoxo macrolide), a compound of formula (I) wherein A and B are taken together and are =O is reduced using standard conditions for the reduction of α -ketoamides. This reduction procedur
20 selectively reduces the carbonyl adjacent to the amide without affecting other carbonyls in the molecule. Generally the C-2 oxo macrolide of formula (I) is dissolved in a reaction inert solvent or mixture of solvents and hydrogen sulfide gas is bubbled through the mixture for 6-24 hours at room temperature. For convenience, the gas is generally bubbled through the reaction mixture overnight. Suitable reaction inert solvents for this
25 reaction include, but are not limited to, organic bases such as diethylamine, triethylamine, dimethylamine, trimethylamine, piperidine, morpholine and aniline; dipolar aprotic solvents such as N,N-dimethylformamide, dimethylsulfoxide and N-methylpyrrolidone; and alcoholic solvents such as methanol, ethanol and propanol. A combination of two or more of these solvents is sometimes used to achieve optimum
30 yield or to affect the course of reduction. For example, the macrolide wherein A is H and B is OH is prepared by using methanol as solvent. A particularly preferred solvent system for providing the C-2 desoxo macrolide is pyridine and N,N-dimethylformamide in equal amounts. When the reaction is completed, the product is isolated using the

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standard techniques of organic chemistry as would be understood by one of ordinary skill in the art.

Alternatively, the macrolide of formula (IV) or (V) can be reduced prior to glycosylation, using the foregoing procedure. Following reduction, the macrolide can
5 be glycosylated as recited hereinabove.

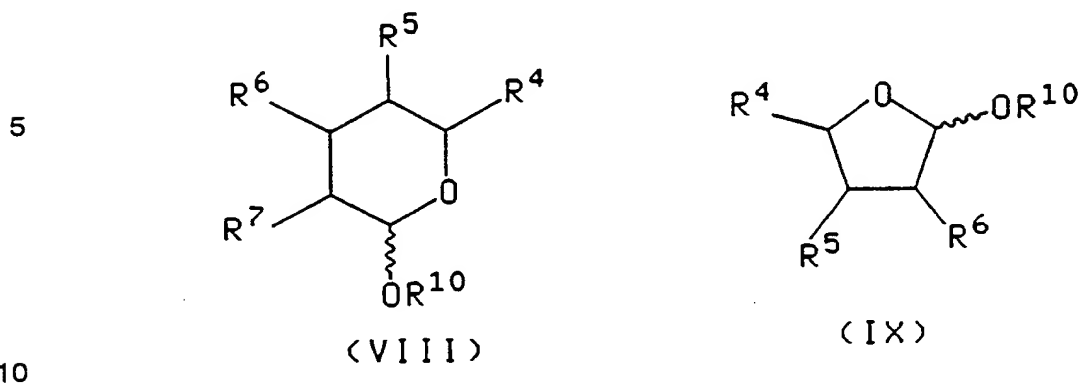
To prepare compounds of the invention of formula (I) wherein the dotted line represents a bond and R^2 is hydrogen, the compound of formula (I) wherein R^2 is -OH and the dotted line represents no bond (hereinafter referred to as the
10 β -hydroxy ketone) is dehydrated as disclosed in European Patent Application No. 323042. Generally the β -hydroxy ketone is dissolved in a reaction inert solvent containing a catalytic amount of an organic acid. Suitable reaction inert solvents are aromatic solvents such as benzene, toluene, xylene and the like, with toluene being preferred. The organic acid is generally selected from such acids as toluenesulfonic acid, camphorsulfonic acid and the like with toluenesulfonic acid being preferred. The
15 reaction mixture is heated at about 50°C to about 120°C for about five minutes to about one hour. Generally steam bath temperatures (about 100°C) are preferred and five minutes is generally sufficient for complete reaction. The reaction product is isolated according to methods well understood by one of ordinary skill in the art. The reaction is generally carried out on compounds which have already been glycosylated.

20 To prepare compounds of the invention of formula (I) wherein R^5 , R^6 and R^7 are hydroxy and R^4 is hydroxymethyl, a compound of formula (I) wherein R^5 , R^6 and R^7 are acetoxy and R^4 is acetoxymethyl is deacetylated using standard conditions known to one of ordinary skill in the art as recited hereinbelow. This selective deacetylation does not affect any amides which are present and is readily accomplished by the addition
25 of an alkoxide base to a solution of the material to be deacetylated in an alcoholic solvent at 0°C. Generally a catalytic amount, such as 0.01 equivalents, of base is used. Usually the alkoxide base of the particular alcoholic solvent in use is preferred. Most preferred, for its ease of use and reactivity, is the system wherein methanol is the solvent and sodium methoxide is the base. Isolation of the product is achieved *via*
30 standard methods well known to those of ordinary skill in the art.

The sugar halide derivatives of formulae (VI) and (VII) are conveniently prepared, when not readily available, from the readily available sugars of formulae (VIII) and (IX)

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wherein R^{10} is H, (C_1-C_4) alkyl or (C_2-C_4) alkanoyl by employing standard methods of halogenation well known to one of ordinary skill in the art.



Bromination is the method of choice; chlorination may also be employed in certain cases. Bromination is effected by dissolving a 1-hydroxy, alkoxy or alkanoyloxy sugar derivative of formula (VIII) or (IX) in an organic acid solvent such as acetic acid. When the sugar is a 1-hydroxy or 1-alkoxy sugar, one to ten equivalents of acetic anhydride are generally added. The preferred substrates are 1-acetoxy sugar derivatives. The reaction mixture is cooled so that the temperature falls within the range of about -20°C to about 0°C . The generally preferred temperature is about 0°C . The cooled reaction mixture is treated with a solution of hydrobromic acid in the acidic solvent. Generally a large excess, such as 10-40 molar equivalents, of hydrobromic acid is employed. The reaction mixture is warmed to room temperature and stirred until the reaction is complete. Generally, for convenience, the reaction mixture is left stirring overnight. The isolation of the brominated product is achieved in a straightforward manner well known to one of ordinary skill in the art. Often this merely involves removing the solvent *in vacuo*. Occasionally, to more fully effect solvent removal, a cosolvent, such as toluene, which azeotropes the reaction solvent is utilized. Further purification is sometimes achieved by the use of column chromatography.

To prepare compounds of formulae (VIII) and (IX) wherein R^4 is $-\text{CH}_2\text{OCOR}^8$; R^5 , R^6 and R^7 are $-\text{OCOR}^8$; and R^{10} is $-\text{COR}^8$, a compound of formulae (VIII) or (IX) wherein R^4 is $-\text{CH}_2\text{OH}$; R^5 , R^6 and R^7 are $-\text{OH}$; and R^{10} is H is used as a substrate in a standard acylation reaction. Typically said substrate is reacted with a suitable acylating agent such as, but not limited to, acetic anhydride, in a suitable solvent such as acetic acid

at about 0°C to about 25°C. The product is isolated utilizing the standard techniques of organic chemistry well known to one of ordinary skill in the art.

The sugar derivatives of formulae (VIII) and (IX) are generally readily available, being for the most part naturally occurring sugars.

5 The sugar derivatives of formula (VIII) and (IX) wherein R⁴ is -COOH are readily available, being naturally occurring uronic acid derivatives of the corresponding sugars. The sugar derivatives of formula (VIII) and (IX) wherein R⁴ is -CONH₂, -CONHR⁸, -CONR₂⁸ or -CO₂R⁸, when not readily available, are readily prepared utilizing well known methods of esterification or amidation known to one of ordinary skill in the art.

10 To prepare the compound of formula (I) wherein R⁴ is -COOH, a sugar halide of formula (VI) or (VII) is coupled with a macrolide of formula (IV) or (V) as described hereinabove. After coupling, the -R⁸ group is removed utilizing the standard de-esterification methods of organic chemistry known to one of ordinary skill in the art, providing a compound of formula (I) wherein R⁴ is -COOH.

15 When the compounds of formula (I) of the present invention are acidic, as when R⁴ is -CO₂H, the invention also embraces pharmaceutically acceptable salts of said compounds of formula (I).

Typical pharmaceutically acceptable cationic salts for such use include alkali metal salts (e.g., sodium and potassium), alkaline earth metal salts (e.g., magnesium
20 and calcium), aluminum salts, ammonium salts and salts with organic amines such as benzathine (N,N'-dibenzylethylenediamine), choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), benethamine (N-benzylphenethylamine), diethylamin , piperazine, tromethamine (2-amino-2-hydroxymethyl-1,3-propanediol) and procaine. An especially preferred such salt is the sodium salt.

25 The pharmaceutically acceptable cationic salts of the present invention are readily prepared by reacting the acid forms with an appropriate base, usually one equivalent, in a co-solvent. Typical bases are sodium hydroxide, sodium methoxide, magnesium hydroxide, calcium hydroxide, benzathine, choline, diethanolamine, piperazine and tromethamine. The salt is isolated by concentration to dryness or by
30 addition of a non-solvent. In many cases, salts are preferably prepared by mixing a solution of the acid with a solution of a different salt of the cation (sodium or potassium ethylhexanoate, magnesium oleate), employing a solvent (e.g., ethyl acetat) from

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which the desired cationic salt precipitates, or can be otherwise isolated by concentration and/or addition of a non-solvent.

With respect to the macrolides of formula (I) of this invention, it is to be understood that there are conformer(s) or stereoisomeric forms such as optical and geometrical isomers due to asymmetric carbon atom(s) and double bond(s), and such isomers are also included within the scope of this invention.

The compounds of formula (I) thus prepared are useful in the treatment of resistance to transplantation and autoimmune diseases such as rheumatoid arthritis or psoriasis. In the treatment of resistance to transplantation, a compound of formula (I) may be used either prophylactically or in response to an adverse reaction by the human subject to a transplanted organ or tissue. When used prophylactically, a compound of formula (I) is administered to the patient or to the tissue or organ to be transplanted in advance of the transplantation operation. Prophylactic treatment may also include administration of the medication after the transplantation operation but before any signs of adverse reaction to transplantation are observed. When administered in response to an adverse reaction, a compound of formula (I) is administered directly to the patient in order to treat said resistance to transplantation after outward signs of the resistance have been manifested.

For use in the treatment of resistance to transplantation and autoimmune diseases such as rheumatoid arthritis or psoriasis in a mammal, including man, a compound of formula (I) is formulated into a suitable pharmaceutical composition containing a disease treating effective amount. Depending upon the potency of the particular compound of formula (I) being administered, about 0.05 mg/kg of body weight per day to about 30 mg/kg of body weight per day, in single or multiple daily doses, is administered to the mammal being treated. A more preferred range is 0.1 mg/kg of body weight per day to about 20 mg/kg of body weight per day, although in particular cases, at the discretion of the attending physician, doses outside the broader range may be required. The preferred route of administration is generally oral, but parenteral administration (e.g., intravenous, intramuscular, subcutaneous or intramedullary) will be preferred in special cases such as where oral administration is inappropriate to the instant target or where the patient is unable for various reasons to ingest the drug. Topical administration may also be indicated, as where the patient is suffering from a skin disease such as psoriasis or whenever the medication is best

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applied to the surface of a tissue or organ as determined by the attending physician.

The compounds of formula (I) thus prepared are also useful in the treatment of infections caused by fungi. For use in the treatment of said fungal infections in a mammal, including man, a compound of formula (I) is formulated into a pharmaceutical composition containing a disease treating effective amount. Depending upon the potency of the particular compound of formula (I) being administered, about 0.05 mg/kg of body weight per day to about 30 mg/kg of body weight per day, in single or multiple daily doses, is the amount administered to the mammal being treated. A more preferred range is 0.1 mg/kg of body weight per day to about 20 mg/kg of body weight per day, although in particular cases, at the discretion of the attending physician, doses outside the broader range may be required. The preferred route of administration is generally oral, but parenteral administration (e.g., intravenous, intramuscular, subcutaneous or intramedullary) will be preferred in special cases such as where oral administration is inappropriate to the instant target or where the patient is unable for various reasons to ingest the drug. Topical administration may also be indicated whenever the medication is best applied to the surface of a tissue or organ as determined by the attending physician.

The compounds of the present invention are generally administered in the form of a pharmaceutical composition comprising at least one of the compounds of formula (I) together with a pharmaceutically acceptable vehicle or diluent. Such compositions are generally formulated in a conventional manner utilizing solid or liquid vehicles or diluents as appropriate to the mode of administration: for oral administration, in the form of tablets, hard or soft gelatin capsules, suspensions, granules, powders and the like; for parenteral administration, in the form of injectable solutions or suspensions and the like; and for topical administration, in the form of solutions, lotions, ointments, salves and the like.

The utility of the compounds of the present invention as medical agents in the treatment of resistance to transplantation and autoimmune diseases such as rheumatoid arthritis or psoriasis is demonstrated by the activity of said compounds in the biological screen described hereinbelow. Said biological screen also provides a means whereby the activities of the compounds of formula (I) can be compared with the activities of other known compounds. The results of these comparisons are useful for determining dosage levels in mammals, including man, for the treatment of

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resistance to transplantation and autoimmune diseases such as rheumatoid arthritis and psoriasis.

The human mixed lymphocyte reaction (MLR) is used to generate an immune response *in vitro* which is measured *via* ^3H -thymidine uptake. This screen uses
5 peripheral blood mononuclear cells in a modified two-way MLR. To ensure disparity of HLA type D antigens and therefore maximize stimulation, a pool of frozen donor cells is used as the stimulator population; freshly isolated cells are used as the responder population.

Freshly drawn mononuclear cells are suspended in RPMI-1640 enriched with:
10 0.5% MEM non-essential amino acids (100x) solution, 1% L-glutamine (200 mM), 1% MEM vitamins (100x), 1% penicillin streptomycin solution (10,000 units/mL) and 15% heat-inactivated human AB serum (NABI). The cells are counted and the concentration is adjusted to 5×10^5 cells/mL. The solution is then transferred to round bottom 96 well plates in 100 μL /well quantities. These plates now contain the responder cells.

15 The stimulator cells are prepared by pooling the mononuclear cells collected from several different individuals. The cells are suspended in 90% human AB serum and 10% DMSO such that the cell count is 2×10^7 cells/mL. The cells are stored in liquid nitrogen. For an MLR, the viable cells are diluted to 5×10^5 cells/mL, and 100 μL /well is added to the plates containing the responder cells. To each well,
20 containing a mixture of responder cells and stimulator cells, is added 50 μL of compound solution. Triplicate wells are run for each dose. The plates are incubated at 37°C under an atmosphere of 5% CO_2 and are humidified for five days. To each well is added 1 μCi of ^3H -thymidine and incubation is continued for another eighteen hours. The cells are harvested using the LKB Beta Plate system.

25 The percent inhibition of stimulated control is obtained using the following equation:

$$\% \text{ Inhibition} = \left[100 - \left(\frac{\text{avg. cpm of drug}}{\text{avg. cpm of stimulated control}} \right) \right] \times 100$$

30

The abbreviation cpm is defined as counts per minute. RPMI-1640 is a tissue culture medium which is available from Sigma.

Activity in the MLR screen recited above is indicative of usefulness of the active compound in the treatment of resistance to transplantation and autoimmune diseases
5 such as rheumatoid arthritis and psoriasis.

Antimicrobial activities of the macrolides of the present invention against various fungi are determined by a serial agar dilution method in a Sabouraud agar. Minimum inhibitory concentrations (MIC) are obtained after incubation for 24 hours at 30°C.

The present invention is illustrated by the following examples. However, it
10 should be understood that the invention is not limited to the specific details of these examples. All reactions are conducted under an inert atmosphere, such as nitrogen, unless otherwise specified. The abbreviations THF, DMSO, DAST, DMAP and Ac, where used, refer to tetrahydrofuran, dimethyl sulfoxide, dimethylamino sulfurtrifluoride, 4-dimethylaminopyridine and acetyl, respectively. The sugar halides were purchased
15 from a reliable vendor such as Sigma or Aldrich, as were the sugars, unless specifically mentioned. Anhydrous solvents were used, anhydrous being defined as substantially free from water.

The expression "reaction inert solvent," where used hereinabove, refers to any solvent which does not interact with starting materials, reagents, intermediates or
20 products in a manner which adversely affects the yield of the desired product.

Terms or acronyms which appear in Preparations 1 and 2 are described in further detail hereinbelow.

PYEA agar is prepared by dissolving Difco maltose (10 g), Difco yeast extract (4 g), dextrose (4 g), Difco agar (15 g) and fresh coconut milk (50 mL) in enough
25 deionized water to yield a one liter solution; and the solution is adjusted to pH 7.3 with 1N NaOH.

ATCC 172 medium is prepared by dissolving glucose (10 g), soluble starch (20 g), yeast extract (5 g), NZ-amine A (Difco, 5 g) and calcium carbonate (1 g) in enough deionized water to yield a one liter solution; and the solution is adjusted to
30 pH 7.0 with 1N KOH.

JDYTT medium is prepared by dissolving cerelose (10 g), corn starch (5 g), corn steep liquor (5 g), NZ-amine YTT (5 g), cobalt chloride (0.002 g) and calcium carbonate

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(3 g) in enough deionized water to yield a one liter solution; and the solution is adjusted to pH 7.2 with 1N NaOH.

NZ-amine A and NA-amine YTT are available from Difco, as are most of the ingredients of the above media.

- 5 In the MLR protocol provided hereinabove, RPMI-1640 is a standard medium for MLR studies; MEM is defined as "minimum essential media"; and NABI is a supplier.

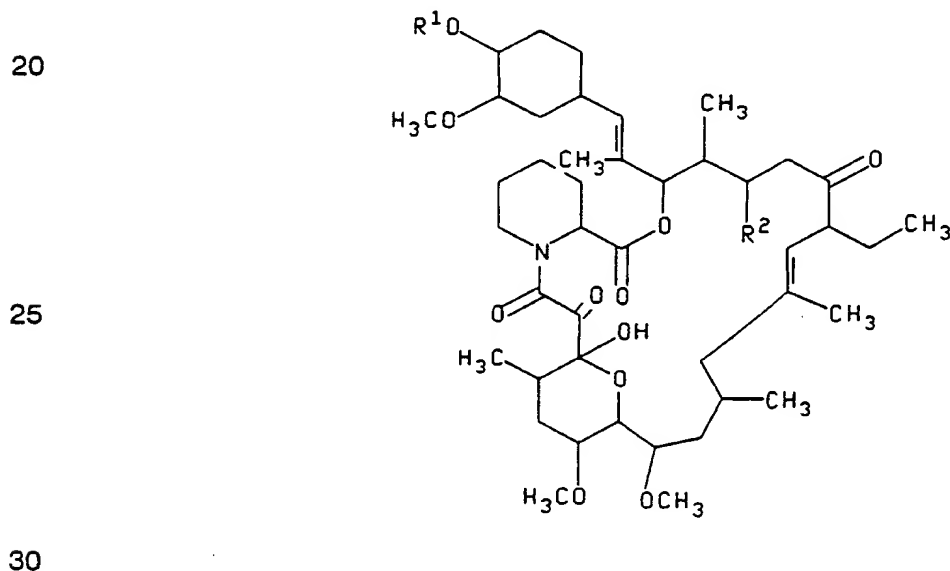
EXAMPLE 1

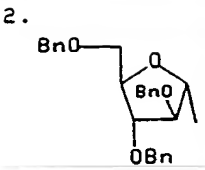
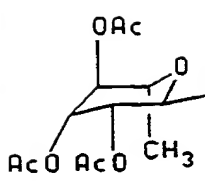
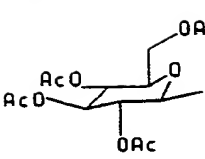
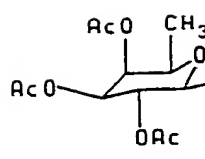
17-Ethyl-1,14-dihydroxy-12-[2'-(4''-(2'''',3''',4'''-tri-O-acetyl- α -D-fucopyranosyloxy)-3''-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0^{4,9}]-octacos-18-ene-2,3,10,16-tetraone

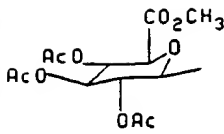
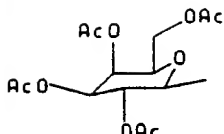
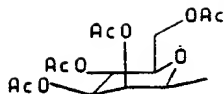
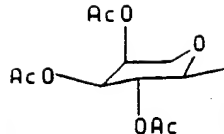
5 To a stirred slurry of the title compound of Preparation 1 (6.41 g, 0.0081 mmol), α -L-acetochlorofucose (Sigma, 5.0 g, 0.016 mmol) and 4Å Molecular sieves (crushed, 5.0 g) in methylene chloride (anhydrous, 150 mL) at -78°C was added silver carbonate (13.36 g, 0.0324 mmol) followed by silver triflate (0.83 g, 0.0032 mmol). The reaction mixture was allowed to warm to room temperature over eight hours and was then
10 stirred for an additional five hours. The resultant tan slurry was filtered through Celite and the filtrate was evaporated *in vacuo*. The residue was purified on silica gel, eluting with hexane/ethyl acetate (2/1) to afford the product as a mixture of anomers (9.0 g, 52%).

EXAMPLES 2-11

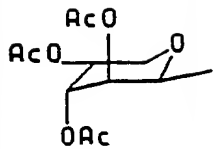
15 By the method of Example 1, but substituting one molar equivalent of the appropriate sugar chloride for each molar equivalent of α -L-acetochlorofucose, the following compounds were prepared.



	<u>R¹</u>	<u>R²</u>	<u>Yield</u>
5	2.		
10		-OH	Mass spectrum (FAB): m/z = 1216.7 (molecular ion + Na ⁺) ¹³ C-NMR: δ 169.0, 164.7, 138.7, 138.4, 138.3, 137.8, 132.5, 123.0
15	3.		
		-OH	Mass spectrum (FAB): m/z = 1086.7 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.4, 196.2, 170.8, 170.2, 170.0
20	4.		
25		-OH	Mass spectrum (FAB): m/z = 1144.7 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.2, 196.3, 171.4 170.5, 170.1, 170.0, 165.1
30	5.		
35		-OH	Mass spectrum (FAB): m/z = 1086.7 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.5, 196.2, 170.7, 170.2, 169.4, 168.9

	<u>R¹</u>	<u>R²</u>	<u>Yield</u>
5	6.	-OH	Mass spectrum (FAB): m/z = 1130.7 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.4, 196.2, 169.4, 169.2, 167.3
10			
15	7.	-OH	Mass spectrum (FAB): m/z = 1144.7 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.2, 196.1, 171.5, 171.2, 170.8, 170.0, 163.1
20			
25	8.	-OH	Mass spectrum (FAB): m/z = 1144.7 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.4, 196.2, 170.8, 170.2, 169.9, 169.2
30			
35	9.	-OH	Mass spectrum (FAB): m/z = 1072.7 (molecular ion + Na ⁺)
			

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	<u>R¹</u>	<u>R²</u>	<u>Yield</u>
5	10.	-OH	Mass spectrum (FAB): m/z = 1072.7 (molecular ion + Na ⁺)
10			

EXAMPLE 12

15 To a stirred solution of the title compound of Example 1 (1.0 g, mmol) in methanol (100 mL) was added sodium methoxide (20 mg, mmol). The reaction mixture was stirred at 0°C for eight hours and then treated with one drop of acetic acid. The solvent was removed *in vacuo* and the residue was purified on silica gel, eluting with THF to afford 610 mg of a foam (72%). Mass spectrum (FAB): m/z = 960.6

20 (molecular ion + Na⁺).

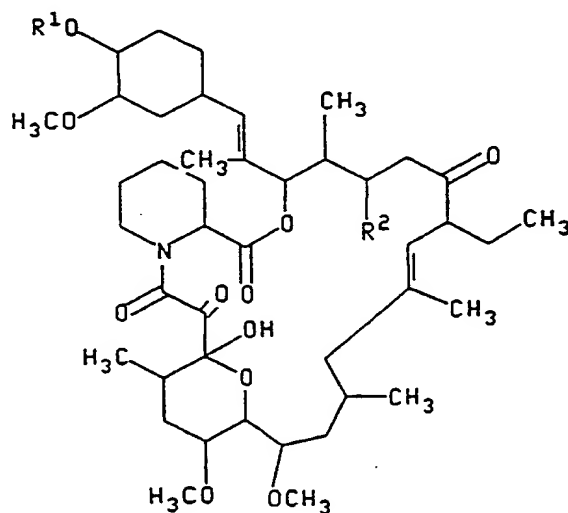
EXAMPLES 13-21

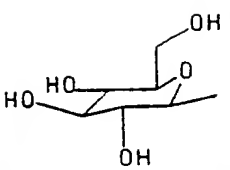
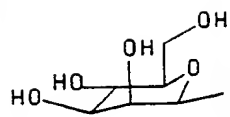
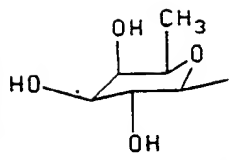
By the method of Example 12, but substituting one molar equivalent of the appropriate title compound of Examples 2-11 for each molar equivalent of the title compound of Example 1, the following compounds were prepared.

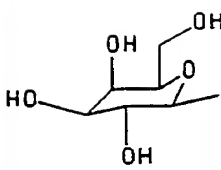
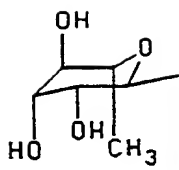
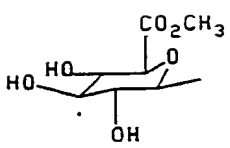
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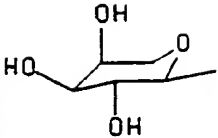
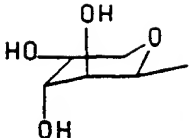
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35



	<u>R¹</u>	<u>R²</u>	<u>Yield</u>
5			
10	13. 	-OH	Mass spectrum (FAB): m/z = 976.6 (molecular ion + Na ⁺) ¹³ C-NMR: δ 212.0, 196.8, 169.3, 166.1, 139.1, 138.5, 132.4, 131.8, 123.5
15			
20	14. 	-OH	Mass spectrum (FAB): m/z = 976.6 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.4, 198.5, 170.6, 167.5, 140.5, 139.8, 133.4, 125.0
25			
30	15. 	-OH	Mass spectrum (FAB): m/z = 960.6 (molecular ion + Na ⁺)

	<u>R¹</u>	<u>R²</u>	<u>Yield</u>
5 16.		-OH	Mass spectrum (FAB): m/z = 976.6 (molecular ion + Na ⁺) ¹³ C-NMR: δ 212.1, 197.0, 169.2, 166.0, 139.1, 138.5, 132.2, 131.8, 131.0, 123.5
10			
15 17.		-OH	Mass spectrum (FAB): m/z = 960.6 (molecular ion + Na ⁺) ¹³ C-NMR: δ 212.0, 197.0, 169.3, 166.7, 166.0, 138.4, 132.0, 131.6, 123.5
20			
25 18.		-OH	Mass spectrum (FAB): m/z = 1004.6 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.4, 197.1, 169.8, 168.4, 166.1
30			

<u>R¹</u>	<u>R²</u>	<u>Yield</u>
19. 	-OH	Mass spectrum (FAB): m/z = 946.5 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.4, 196.2, 169.0, 164.7, 138.7, 137.8, 132.4
20. 	-OH	Mass spectrum (FAB): m/z = 946.5 (molecular ion + Na ⁺) ¹³ C-NMR: δ 213.2, 196.0, 168.8, 165.0, 138.5, 137.8, 133.1

EXAMPLE 21

17-Ethyl-1,14-dihydroxy-12-[2'-(4'-(2''',3''',4'''-tri-O-acetyl- α -D-rhamnopyranosyloxy)-3''-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.O^{4,9}]-octacos-18-ene-3,10,16-trione

- 5 The title compound of Example 3 (1.0 g) was dissolved in DMF (15 mL) and pyridine (15 mL) and hydrogen sulfide gas was bubbled through the reaction mixture for 16 hours at room temperature. Excess hydrogen sulfide was prevented from escaping into the atmosphere by means of a Chlorox™ trap. After 16 hours, the reaction mixture was diluted with toluene (50 mL) and washed with brine (50 mL). The solvent
- 10 was dried with MgSO₄ and the solvent was removed *in vacuo*. The residue was passed through a pad of silica gel and eluted with ethyl acetate/hexane (2/1) to afford the title compound. Mass spectrum (FAB): m/z = 1072.7 (molecular ion + Na⁺). ¹³C-NMR: δ 215.3, 174.0, 170.3, 170.1, 169.4, 141.1 and 132.6.

PREPARATION 1

17-Ethyl-1,14-dihydroxy-12-[2'-(4"-hydroxy-3"-methoxy-cyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.O^{4,9}]-octacos-18-ene-2,3,10,16-tetraone

- 5 *Streptomyces hygroscopicus* subsp. *ascomyceticus* culture ATCC 14891 was carried on PYEA agar slants (10 g/L of Difco maltose, 4 g/L of Difco yeast extract, 4 g/L of dextrose, 15 g/L of Difco agar and 50 mL of fresh coconut milk which was diluted up to one liter with deionized water, then adjusted to pH 7.3 with 1N NaOH). The preparation was incubated for 10 to 12 days at 28°C, and the spores were then
- 10 transferred to sterile 1x6 shake tubes containing 10 mL of ATCC 172 medium (10 g/L of glucose, 20 g/L of soluble starch, 5 g/L of yeast extract, 5 g/L of NZ-amine A and 1 g/L of calcium carbonate. The pH was adjusted to 7.0 with 1N KOH). The tubes were incubated at 28°C and shaken on a rotary shaker at about 150 to 200 cycles/minute. After 4 to 5 days the broth was diluted to 40% with glycerol and
- 15 ATCC 172 medium and then transferred aseptically to cryotubes. Each tube was charged with 1/2 mL of broth. The tubes were frozen at -80°C during storage.

- The tubes from the ultra cold stock were used as seed inoculum for the preparation of inoculum flasks, one tube per 50 mL of sterile JDYTT medium in 300 mL shake flasks. The composition of the JDYTT medium was 10 g/L of cerelose,
- 20 5 g/L of NZ-amine YTT, 0.002 g/L of cobalt chloride and 3 g/L of calcium carbonate. The pH of the JDYTT medium was adjusted to pH 7.2 with 1N NaOH. The shake flasks were shaken and incubated on a rotary shaker at about 150-200 cycles/minute and 28°C.

- Two mL of an about 3 to 5 day-old shake flask inoculum was used to
- 25 inoculate the second stage flask inoculum containing 80 mL of JDYTT medium in a 3L jar fermenter. The fermenter medium was 45 g/L of corn starch, 10 g/L of corn steep liquor, 10 g/L of amber or Baker dried yeast, 3 g/L of calcium carbonate and 0.005 g/L of cobalt chloride. The pH was adjusted to about 6.4 to 6.8 with 1N NaOH. One mL of antifoam P-2000 was added to the jar fermenters together with 100 mL of
- 30 soya bean oil. The pH was adjusted to about 6.4 to 6.8 with 1N NaOH and the material was agitated at 1700 rpm. The temperature was maintained at 28°C and sterile air was sparged through the medium at the rate of one volum per volume per minute.

After inoculation, sterile soya bean oil was used to control foaming. In longer fermentations, and, depending on media used, the sugar content can be monitored and

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sugar feeds used at 40, 60 and 90 hours to maintain the reducing sugar level at or above 0.05%. The fermentation was run for 46 hours.

Using standard methods of thin-layer chromatography and HPLC, the fermentation broth was monitored and relative potency was calculated.

5 The product was found primarily in the mycelium, but workup of the whole broth is preferred. Thus, after the fermentation has run its course, the whole broth was extracted twice with one-third to one-half of its volume of methylisobutylketone (MIBK). The layers were separated by means of a DeLaval separator or a Podbielnack extractor. The solvent layer was clarified and concentrated first in a vacuum pan and then in a
10 rotary evaporator. The concentrate was subjected to four tube counter current distribution in 20 liter carbuoys using 10 liter top layer and 1 liter bottom layer per carbuoy of a heptane/acetonitrile 10/1 system. The active bottom layers were collected, combined and concentrated. The material was further purified *via* filtration through Florisil (washing with hexane, hexane/methylene chloride and methylene chloride,
15 successively, with a gradual increase in methylene chloride). Most of the activity was found in the methylene chloride fractions. These were combined and concentrated. A second filtration step was performed, this time through silica gel (washing with heptane, methylene chloride, methylene chloride/ethyl acetate and ethyl acetate). The activity was mostly found in the fractions containing a methylene chloride/ethyl acetate
20 mixture and the fractions containing only ethyl acetate. These were combined and concentrated, redissolved in methylene chloride and treated with DARCO G60. The sample was then divided into 12 to 15 g portions and each sample was further chromatographed on a Prep 500 liquid chromatograph using silica gel columns and eluting using a linear gradient beginning with 100% methylene chloride and ending with
25 100% ethyl acetate. The active cuts were combined, concentrated and chromatographed on a Prep 500, using reversed phase (¹⁸C) silica gel and eluting with a linear gradient beginning with acetone and ending with 100% water. Clean product was obtained as the last component isolated off the column.

30 The active fractions in the foregoing fermentation procedure were determined using the following bioassay.

A 12.5 mm disc was applied directly to the agar surface. *Candida albicans* ATCC 14053, *Saccharomyces pastorianus* FD3737 and a sensitive strain of *Byssoschlamys fulva* FM 10,300(S) and FM 10,464(R) were used. The *Candida* and

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Saccharomyces plates were incubated at 37°C for 18 hours, then the plates were examined for activity. The *Byssoschlamys* plates were incubated at 28°C and read after 18 hours. Plates containing only FK506 and FK520 (CP-105051) were active against the *Byssoschlamys* strain. Impure fractions (containing nigericin) were active against the other strains as well.

An HPLC method for determining the purity of the fractions was also used. The method entailed using a Dupont Zorbax CN column (4.6 mm x 25 cm) and an isocratic system composed of 55/45 water/acetonitrile and a flow rate of one mL/min. Detection was accomplished at 214 nm. The broth sample (20 mL) was mixed with MIBK (20 mL) and shaken for about 4 to 5 minutes. The layers were separated and the solvent was concentrated to near dryness. The residue was taken up in 1 mL of neat acetonitrile and a 5 µL sample was injected into the HPLC. The retention time for FK520 is approximately 12.7 minutes under these conditions.

PREPARATION 2

15 17-Allyl-1,14-dihydroxy-12-[2'-(4"-hydroxy-3"-methoxy-cyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.O^{4,9}]-octacos-18-ene-2,3,10,16-tetraone

Streptomyces tsukubaensis No. 9993 FERM BP-927 was carried on PYEA agar slants (10 g/L of Difco maltose, 4 g/L of Difco yeast extract, 4 g/L of dextrose, 15 g/L of Difco agar and 50 mL of fresh coconut milk which was diluted up to one liter with deionized water, then adjusted to pH 7.3 with 1N NaOH). The preparation was incubated for 10 to 12 days at 28°C, and the spores were then transferred to sterile 1x6 shake tubes containing 10 mL of ATCC 172 medium (10 g/L of glucose, 20 g/L of soluble starch, 5 g/L of yeast extract, 5 g/L of NZ-amine A and 1 g/L of calcium carbonate. The pH was adjusted to 7.0 with 1N KOH). The tubes were incubated at 28°C and shaken on a rotary shaker at about 150 to 200 cycles/minute. After 4 to 5 days the broth was diluted to 40% with glycerol and ATCC 172 medium and then transferred aseptically to cryotubes. Each tube was charged with 1/2 mL of broth. The tubes were frozen at -80°C during storage.

The tubes from the ultra cold stock were used as seed inoculum for the preparation of inoculum flasks, one tube per 50 mL of sterile JDYTT medium in 300 mL shake flasks. The composition of the JDYTT medium was 10 g/L of cerelose, 5 g/L of NZ-amine YTT, 0.002 g/L of cobalt chloride and 3 g/L of calcium carbonate. The pH of the JDYTT medium was adjusted to pH 7.2 with 1N NaOH. The shake flasks

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were shaken and incubated on a rotary shaker at about 150-200 cycles/minute and 28°C.

Two mL of an about 3 to 5 day-old shake flask inoculum was used to inoculate the second stage flask inoculum containing 80 mL of JDYTT medium in a
5 3L jar fermenter. The fermenter medium was 45 g/L of corn starch, 10 g/L of corn steep liquor, 10 g/L of amber or Baker dried yeast, 3 g/L of calcium carbonate and 0.005 g/L of cobalt chloride. The pH was adjusted to about 6.4 to 6.8 with 1N NaOH. One mL of antifoam P-2000 was added to the jar fermenters together with 100 mL of soya bean oil. The pH was adjusted to about 6.4 to 6.8 with 1N NaOH and the material
10 was agitated at 1700 rpm. The temperature was maintained at 28°C and sterile air was sparged through the medium at the rate of one volume per volume per minute.

After inoculation, sterile soya bean oil was used to control foaming. In longer fermentations, and, depending on media used, the sugar content can be monitored and sugar feeds used at 40, 60 and 90 hours to maintain the reducing sugar level at or
15 above 0.05%. The fermentation was run for 46 hours.

Using standard methods of thin-layer chromatography and HPLC, the fermentation broth was monitored and relative potency was calculated.

The fermenters were stopped and extracted twice with ½ its volume of methylisobutylketone (MIBK). The solvent layer was separated by aspiration and
20 concentration *in vacuo* to a viscous oil. The oil was triturated with hexane, diethyl ether and methylene chloride and the active cuts (the diethyl ether cuts) were chromatographed on florisil. The florisil was eluted with, successively, diethyl ether methylene chloride, ethyl acetate and acetone. The eluate was concentrated and treated with activated charcoal. The concentrate was filtered and dissolved in ethyl
25 acetate. Hexane was added to crystallize the product.

The bioactivity of the broth and subsequent recovery streams was followed by using a strain of *Byssoschlamys fulva*. The components in the broth and recovery streams were visualized by chromatography on Analtech silica gel GF (Trademark) plates using neat ethyl acetate as the eluant. The developed plates were sprayed with
30 vanillin reagent (3 g of vanillin in 75 mL of ethanol and 25 mL of 85% phosphoric acid) and heated to 80°C. The product appeared as a violet spot.

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PREPARATION 32,3,5-Tri-O-benzyl- β -arabinofuranosyl Bromide

2,3,5-Tri-O-benzyl-1-O-p-nitrobenzoyl- β -arabinofuranoside (Sigma, 5.69 g, 10 mmols) was dissolved in methylene chloride (25 mL) at 0°C and treated with 33% HBr in acetic acid (Fluka, 25 mL). The mixture was stirred at room temperature for two hours. The solvent was removed *in vacuo* and the residue was azeotroped from toluene to afford a brownish oil. Yield 98% used as is.

PREPARATIONS 4-6

By performing the procedures described by Kartha, K.P.R. and H. J. Jennings, Journal of Carbohydrate Chemistry, 9, 777-781 (1990), the following compounds were prepared.

4. 2,3,4-Tri-O-acetyl- β -arabinosyl bromide
5. 2,3,4-Tri-O-acetyl- α -arabinosyl bromide
6. 2,3,4-Tri-O-acetyl- β -rhamnosyl bromide

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CLAIMS

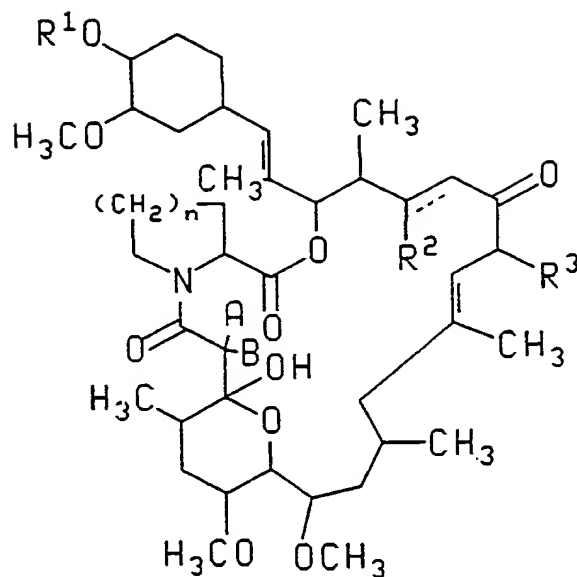
1. A compound of the formula

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or a pharmaceutically acceptable salt thereof;

wherein n is 1 or 2;

the dotted line represents an optional double bond in the case where R^2 is H;

- 25 A and B are taken separately and are each independently H or OH, or A and B are taken together and form $=O$;

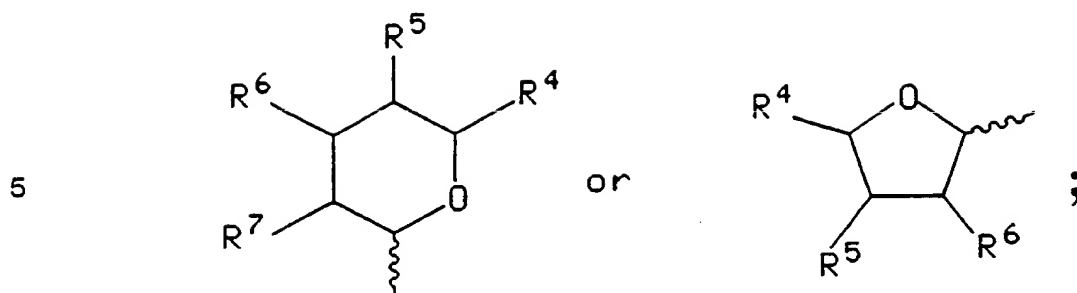
R^2 is H, (C_2-C_5) alkanoyloxy or $-OR^0$;

R^3 is $(C_1$ to $C_3)$ alkyl or allyl;

R^1 and R^0 are each H,

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R^4 is, for each occurrence, independently $-\text{CO}_2R^8$, $-\text{CO}_2\text{H}$, $-\text{CH}_2\text{OH}$, H , $-\text{CH}_3$, $-\text{CONH}_2$, $-\text{CONHR}^8$, $-\text{CONR}_2^8$, $-\text{CH}_2\text{OCOR}^8$, $-\text{CH}_2\text{OCO}_2R^8$, $-\text{CH}_2\text{OCONHR}^8$, $-\text{CH}_2\text{OCONR}_2^8$ or $-\text{CH}_2\text{OR}^8$;

R^5 , R^6 and R^7 are, for each occurrence, independently $(\text{C}_1 \text{ to } \text{C}_4)\text{alkoxy}$, benzyloxy, $-\text{OH}$, $-\text{OCOR}^8$, $-\text{OCO}_2R^8$ or $-\text{OSi}(\text{R}^9)_3$;

R^8 is $(\text{C}_1 \text{ to } \text{C}_6)\text{alkyl}$, $(\text{C}_3 \text{ to } \text{C}_6)\text{cycloalkyl}$, allyl, pyridyl, thienyl, benzyl, benzyl variously substituted with one to five halogen atoms, $-\text{OH}$ groups, or $(\text{C}_1 \text{ to } \text{C}_4)\text{alkoxy}$ groups, phenyl or phenyl variously substituted with one to five halogen atoms, $-\text{OH}$ groups or $(\text{C}_1 \text{ to } \text{C}_4)\text{alkoxy}$ groups; and

R^9 is, for each occurrence, independently $(\text{C}_1 \text{ to } \text{C}_4)\text{alkyl}$, phenyl or benzyl; provided that R^1 and R^0 are not both H .

2. A compound according to claim 1 wherein the dotted line represents no bond and R^2 is $-\text{OH}$.

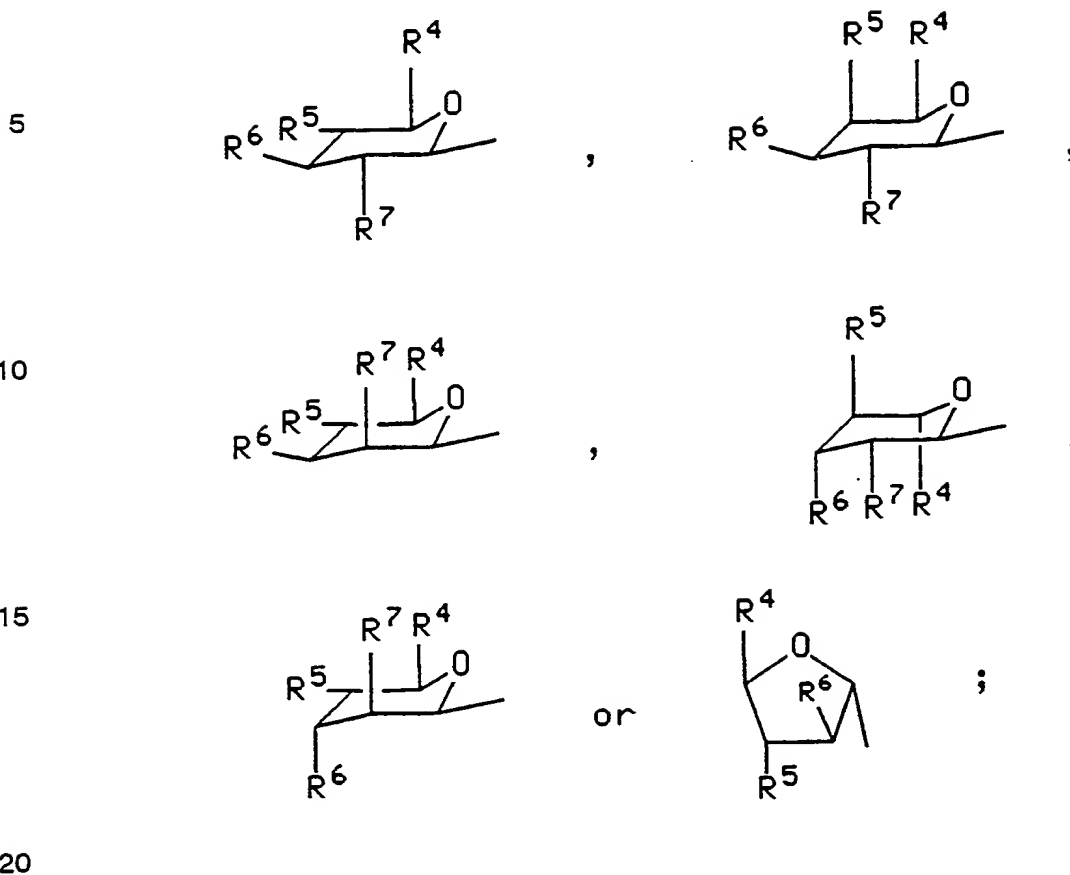
3. A compound according to claim 2 wherein n is 2 and A and B are taken together and form $=\text{O}$.

4. A compound according to claim 3 wherein R^3 is methyl, ethyl or allyl.

5. A compound according to claim 4 wherein R^3 is ethyl.

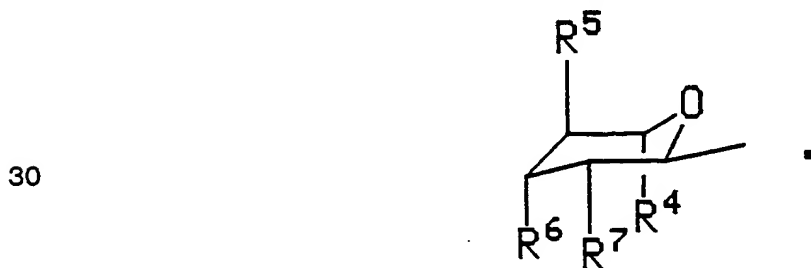
-36-

6. A compound according to claim 5 wherein R¹ is



7. A compound according to claim 6 wherein R⁴ is
 -CH₂OH, -CH₃, -CH₂OCOCH₃, -CH₂OCOCH₂C₆H₅ or -CO₂CH₃; and R⁵, R⁶ and R⁷ are
 25 each, independently, -OH, OCOCH₃ or -OCOCH₂C₆H₅.

8. A compound according to claim 7 wherein R¹ is

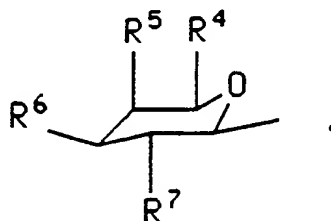


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9. The compound according to claim 8 wherein R^4 is $-\text{CH}_3$, R^5 is $-\text{OCOCH}_3$, R^6 is $-\text{OCOCH}_3$ and R^7 is $-\text{OCOCH}_3$.

10. The compound according to claim 8 wherein R^4 is $-\text{CH}_3$, R^5 is $-\text{OH}$, R^6 is $-\text{OH}$ and R^7 is $-\text{OH}$.

5 11. A compound according to claim 7 wherein R^1 is



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12. The compound according to claim 11 wherein R^4 is $-\text{CH}_2\text{OCOCH}_3$ and R^5 , R^6 and R^7 are each $-\text{OCOCH}_3$.

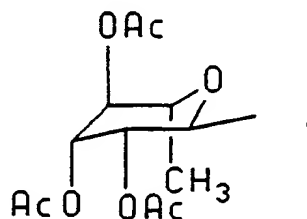
13. The compound according to claim 11 wherein R^4 is $-\text{CH}_2\text{OCOCH}_3$ and R^5 , R^6 and R^7 are each $-\text{OH}$.

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14. A compound according to claim 1 wherein n is 2; A and B are taken separately and are each H; the dotted line represents no bond; R^2 is $-\text{OH}$ and R^3 is ethyl.

15. The compound according to claim 14 wherein R^1 is

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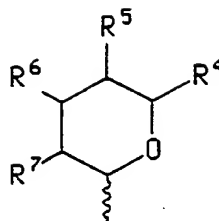


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16. A compound according to claim 1 wherein n is 2; A and B are taken together and form $=\text{O}$; the dotted line represents no bond; R^2 is OR^0 ; R^3 is ethyl and R^0 and R^1 are each

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10 17. A method for treating resistance to transplantation in a mammal in need of such treatment comprising administering to said mammal a resistance to transplantation treating effective amount of a compound according to claim 1 or a pharmaceutically-acceptable salt thereof.

15 18. A method for treating autoimmune disease in a mammal in need of such treatment comprising administering to said mammal an autoimmune disease treating effective amount of a compound according to claim 1 or a pharmaceutically-acceptable salt thereof.

20 19. A method for treating fungal diseases in a mammal in need of such treatment comprising administering to said mammal a fungal disease treating effective amount of a compound according to claim 1 or a pharmaceutically-acceptable salt thereof.

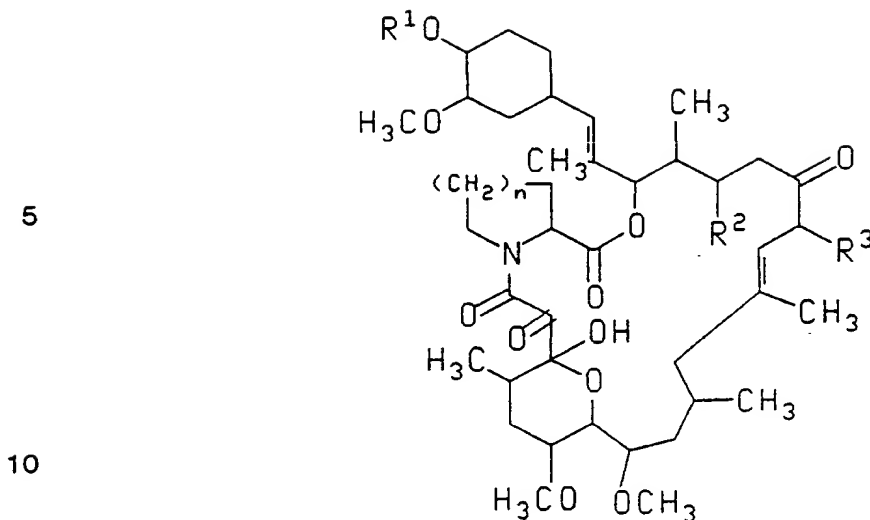
20 20. A pharmaceutical composition comprising a resistance to transplantation treating effective amount of a compound of claim 1 and a pharmaceutically-acceptable carrier.

25 21. A pharmaceutical composition comprising an autoimmune disease treating effective amount of a compound of claim 1 and a pharmaceutically-acceptable carrier.

25 22. A pharmaceutical composition comprising a fungal disease treating effective amount of a compound of claim 1 and a pharmaceutically-acceptable carrier.

30 23. A process for preparing a compound of the formula

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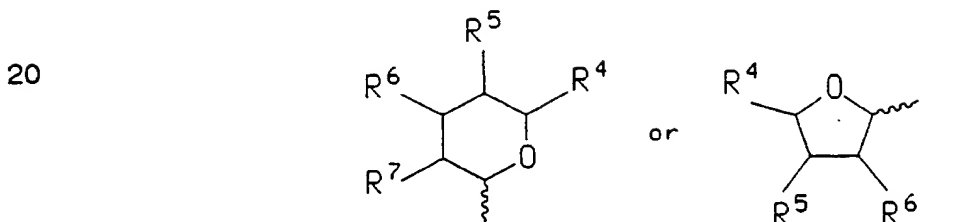


wherein n is 1 or 2;

15 R^2 is (C_2-C_5) alkanoyloxy or $-OR^0$;

R^3 is $(C_1 \text{ to } C_3)$ alkyl or allyl;

R^1 and R^0 are each H,



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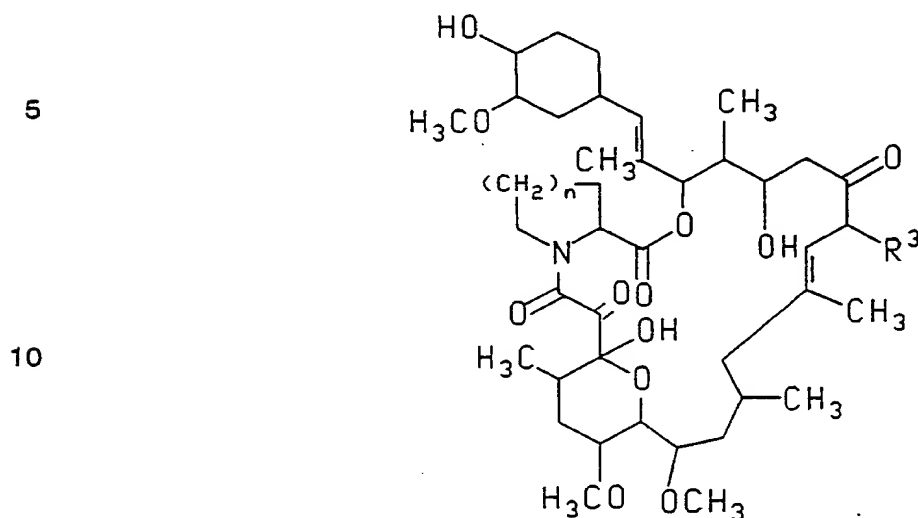
R^4 is, for each occurrence, independently $-CO_2R^8$, $-CO_2H$, H, $-CH_3$, $-CONH_2$, $-CONHR^8$, $-CH_2OCOR^8$, $-CH_2OCO_2R^8$, $-CH_2OCONR_2^8$, $-CH_2OCONR_2^8$ or $-CH_2OR^8$;

R^5 , R^6 and R^7 are, for each occurrence, independently $(C_1 \text{ to } C_4)$ alkoxy, benzyloxy, $-OCOR^8$, $-OCOCH_2R^8$, or $-OSi(R^8)_3$;

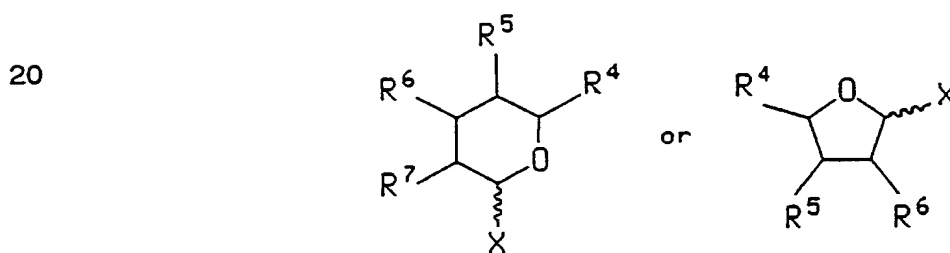
30 R^8 is (C_1-C_6) alkyl, (C_3-C_6) cycloalkyl, allyl, pyridyl, thienyl, benzyl, benzyl variously substituted with one to five halogen atoms, $-OH$ groups or $(C_1 \text{ to } C_4)$ alkoxy groups, phenyl or phenyl variously substituted with one to five halogen atoms, $-OH$ groups or $(C_1 \text{ to } C_4)$ alkoxy groups; and

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R^9 is, for each occurrence, independently (C_1 to C_4)alkyl, phenyl or benzyl;
provided that R^1 and R^0 are not both H, comprising reacting a compound of the formula



wherein R^3 is (C_1 to C_3)alkyl or allyl and n is 1 or 2 with 2 to 4 molar equivalents of a compound selected from the group consisting of



wherein X is halo;

R^4 is, for each occurrence, independently $-CO_2R^8$, $-CO_2H$, H, $-CH_3$, $-CONH_2$, $-CONHR^8$, $-CONR_2^8$, $-CH_2OCOR^8$, $-CH_2OCO_2R^8$, $-CH_2OCONHR^8$, $-CH_2OCONR_2^8$ or $-CH_2OR^8$;

30 R^5 , R^6 and R^7 are, for each occurrence, independently (C_1 to C_4)alkoxy, benzyloxy, $-OCOR^8$, $-OCO_2R^8$ or $-OSi(R^9)_3$;

R^8 is (C_1 to C_6)alkyl, (C_3 to C_6)cycloalkyl, allyl, pyridyl, thienyl, benzyl, benzyl variously substituted with one to five halogen atoms, $-OH$ groups or (C_1 to C_4)alkoxy groups,

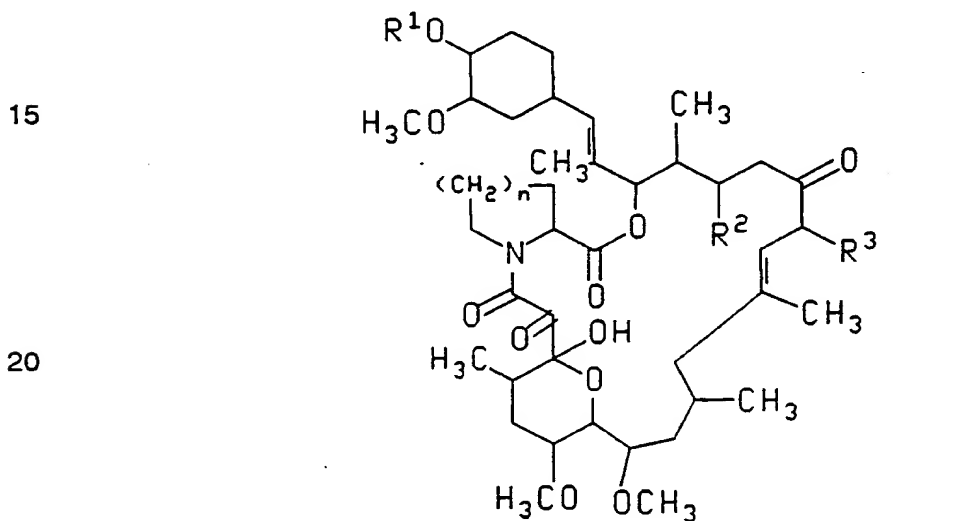
-41-

phenyl or phenyl variously substituted with one to five halogen atoms, -OH groups or (C₁ to C₄)alkoxy groups; and

R⁹ is, for each occurrence, independently (C₁ to C₄)alkyl, phenyl or benzyl, in the presence of a drying agent selected from the group consisting of molecular sieves,

5 calcium sulfate and magnesium sulfate; a base selected from the group consisting of mercuric carbonate, silver carbonate, mercuric nitrate and silver nitrate; and a catalyst selected from the group consisting of silver triflate, silver perchlorate, silver tetrafluoroborate, mercuric triflate, mercuric perchlorate and mercuric tetrafluoroborate in a reaction inert solvent at about -78°C to about -70°C with warming to about 0°C
10 for about 0.5 to about 24 hours and subsequent stirring at room temperature for about 0.5 to 24 hours.

24. A process for preparing a compound of the formula



wherein n is 1 or 2;

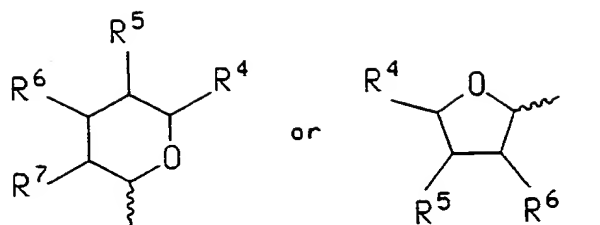
R² is (C₂-C₅)alkanoyloxy or -OR⁰;

R³ is (C₁ to C₃)alkyl or allyl;

R¹ and R⁰ are each H,

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R⁴ is, for each occurrence, independently -CO₂R⁸, -CO₂H, -CH₂OH, H, -CH₃, -CONH₂, -CONR₂⁸, -CH₂OCO₂R⁸, -CH₂OCONHR⁸, -CH₂OCONR₂⁸ or -CH₂OR⁸;

10 R⁵, R⁶ and R⁷ are, for each occurrence, independently (C₁ to C₄)alkoxy, benzyloxy, -OH, -OCO₂R⁸ or -OSi(R⁸)₃;

R⁸ is (C₁-C₆)alkyl, (C₃-C₆)cycloalkyl, allyl, pyridyl, thienyl; benzyl, benzyl variously substituted with one to five halogen atoms, -OH groups or (C₁-C₄)alkoxy groups, phenyl or phenyl variously substituted with one to five halogen atoms, -OH groups or (C₁-

15 C₄)alkoxy groups; and

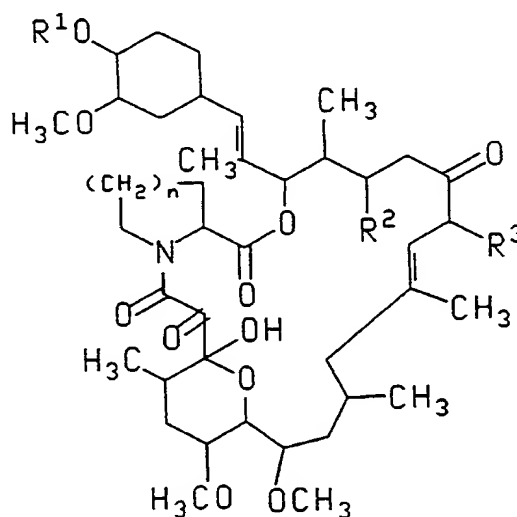
R⁹ is, for each occurrence, independently (C₁ to C₄)alkyl, phenyl or benzyl;

provided that R¹ and R⁰ are not both H, comprising reacting a compound of the formula

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wherein n is 1 or 2;

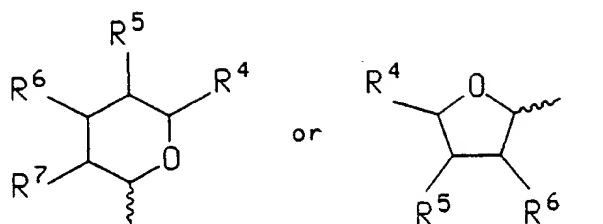
-43-

R^2 is (C_2-C_5) alkanoyloxy or $-OR^0$;

R^3 is $(C_1 \text{ to } C_3)$ alkyl or allyl;

R^1 and R^0 are each H,

5



10

R^4 is, for each occurrence, independently $-CO_2R^8$, $-CO_2H$, H, $-CH_3$, $-CONH_2$, $-CONHR^8$, $-CH_2OCOR^8$, $-CH_2OCO_2R^8$, $-CH_2OCONR_2^8$, $-CH_2OCONR_2^8$ or $-CH_2OR^8$;

R^5 , R^6 and R^7 are, for each occurrence, independently $(C_1 \text{ to } C_4)$ alkoxy, benzyloxy,

15 $-OCOR^8$, $-OCOCH_2R^8$, or $-OSi(R^8)_3$;

R^8 is (C_1-C_6) alkyl, (C_3-C_6) cycloalkyl, allyl, pyridyl, thienyl, benzyl, benzyl variously substituted with one to five halogen atoms, $-OH$ groups or $(C_1 \text{ to } C_4)$ alkoxy groups, phenyl or phenyl variously substituted with one to five halogen atoms, $-OH$ groups or $(C_1 \text{ to } C_4)$ alkoxy groups; and

20 R^9 is, for each occurrence, independently $(C_1 \text{ to } C_4)$ alkyl, phenyl or benzyl;

provided that R^1 and R^0 are not both H, with a catalytic amount of an alkoxide base in an alcohol solvent at $0^\circ C$.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/00470

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate)		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 C07H19/01; A61K31/70		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C07H ; C07D ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X,Y	EP,A,0 466 365 (MERCK AND CO. INC.) 15 January 1992 see page 11 - page 12	1,17-22
Y	EP,A,0 323 042 (FISONS PLC) 5 July 1989 see page 18 - page 21	1,17-22
<p>¹⁰ Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 17 MAY 1993		Date of Mailing of this International Search Report 01.06.93
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer DAY G.J.

Form PCT/ISA/210 (second sheet) (January 1985)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/00470

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 17-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. (PCT-Rule 39.1(iv))
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9300470
SA 69812

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 17/05/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0466365	15-01-92	CA-A- 2046040	04-01-92
		JP-A- 4230399	19-08-92

EP-A-0323042	05-07-89	AU-A- 2822889	05-07-89
		EP-A- 0346427	20-12-89
		WO-A- 8905304	15-06-89
		JP-T- 2502463	09-08-90

EPO FORM P0079

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82